Studies on lipase productivity of *Candida albicans* and *Saccharomyces cerevisiae* using cheap substrates

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SUMMARY

In the presents study the lipase-producing organism were isolated from the soil sample using olive oil containing medium. The isolates were identified based on the morphological and biochemical characteristics. The isolated *Candida albicans* and *Saccharomyces cerevisiae* were inoculated in to inoculam medium and incubated at 37°C for 24hours used as the inoculum. The inoculums mixed with fermentative substrate like molasses and soybean, after incubation, the lipase was estimated. In the study high lipase activity (0.31±µ/ml/min) was observed in *Candida albicans* inoculated medium. Effect of physiochemical parameter was analyzed for optimum enzyme productivity in this study maximum productivity was noted in pH-7 and temperature 35-40°C. Lipase productivity also studied in the free immobilized cell. In this study lipase productivity was maximum noted in the immobilized cell (0.51± 0.09µ/ml/min).

Key words : *Candida albicans*, *Saccharomyces cerevisiae aereus*, Olive oil.

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Lipase has become one of the prominent industrial enzymes for their specificity in hydrolysis and interesterification. They catalyze both the hydrolysis of triglycerides and the synthesis of esters from glycerol and long chain fatty acids (Benjamin and pandy, 1997). In addition, they also serve as biocatalyst for alcoholysis, acidolysis, esterification and aminoacids (Dai *et al*., 2000). Lipase is produced by various microbes, such as bacteria, fungi, yeast, and also in the pancreas of mammals, like pigs and humans. They have also been reported in higher plants, such as castor bean (*Ricinus communis*) and rapeseed (*Brassica napus*) (Elad *et al.*, 1982).

Numerous lipases have been characterized and efforts have been made to improve their stability in organic solvents for varied applications (Elibol and Ozer, 2000; Faird *et al.*, 1994). The most important commercial use of lipases was added to 13 billion tonEs of detergents produced every year (Benjamin and pandy, 1997). Lipase are also emerging as important enzymes in the field of biopolymers. They are used in the synthesis of polymers (Faird *et al.*, 1994). Immobilized *Pseudomonas fluorescence* lipase has been used for the production of bio diesel fuel from triglycerides and alcohols (Fukuda *et al.*, 2001).

Transesterification of oils catalyzed by lipase have fuel (Gross *et al*., 2001). Use of organic solvents in transesterification reactions by lipase in producing methyl esters from sunflower oil showed improved conversion (Faird *et al.*, 1994). Another major industrial application of lipases is in resolving racemixtures (Hellyer *et al.*, 1999; Hung *et al.*, 2003; Kierstan and Bucke, 1977). Optimization of the enantioselective resolution reactions in various bioreactors, like biphasic enzyme membrane reactors (Nawani *et al*., 1998) and packed bed reactors (Paloma *et al.*, 2003) which favours large-scale production. Applications of lipases also extend to the field of waste management and improving tanning technique (Pandey *et al*., 1999) and in separation, which are difficult-to-separate mixtures of organic acids (Sakakai *et al.*, 2001; Jaeger and Reetz, 1998; Kamal *et al.*, 2002).

MATERIALS AND METHODS

One gram of soil sample was suspended in 10ml sterile water. After shaking, 5ml suspension was added in 250ml Erlenmeyer flask containing 25ml of enrichment medium. The medium was incubated at 30°C on a rotator shaker at 200 rev / min for 3-5days, and then aliquot was transferred to fresh medium and cultured again under the same condition. The above incubation and transfer operation were repeated for 5 to 6 times until microbial cells in the culture became nearly uniform (same were periodically observed under microbes). The enrichment cultivation on olive oil was carried out on the assumption that

microorganism capable of growth on olive oil are capable of producing lipases; and high alkaline pH was employed on the assumption that only bacteria could grow under this condition.

The grow microorganism in the enrichment culture were isolated on nutrient agar plates containing olive oil (2.5%) and Victoria Blue B (4mg/100ml) with an initial pH of 9.5-10.0, growing colony with blue colour zone were isolated and subsequently screened by the double layer tributyrinr agar method of colony with large clear zone were collected. Subsequent screening test were then made recording lipase fermentation activity.

In this study molasses and soybean extract used as a medium for fermentation the best solid substrate achieved by this step was fixed in subsequent experiment.

Agar slat of pure culture were used as source of preparing inoculums Erlenmeyer flask (250) contain 50ml of medium and incubated at 35°C an incubator shaker at 250 rpm for 16hrs. One loop from the nutrient agar slant was inoculated 30ml nutrient broth medium and incubated at 30^0C on the rotatory shaker at 150 rev / min for 24h. 1ml of this pre culture fluid was inoculated in 250 ml Erlenmeyer flask containing 100ml of each substrate medium with initial pH 7. The mixture was incubated in rotary shaking for 72 hrs; the lipase activity of the culture broth supernatant (centrifuged at 3000 rev/min for 20min) was measured.

The estimation of lipase secreted by the antagonists in culture (Sanchez *et al*., 2000) Crude enzyme (2ml) was diluted with 8ml of distilled water and mixed well with 500ìl of vegetable oil. This reaction mixture was incubated at 37°C for 2h in a rotary shaker (200rpm). Ethanol was added to it to get a final concentration of 30%. Free fatty acids were extracted with 25ml of pure petroleum ether and the extract was evaporated in a rotary evaporator. The free fatty acids were dissolved in 15ml of neutralized ethanol containing phenolphthalein at 60°C. Each sample was titrated with ethyl alcohol containing 0.5 N NaOH. Free fatty acids were neutralized and one lipolytic unit (LU) was defined as micromoles of NaOH / mg portion/h.

Optimizing the initial pH of the basal medium the pH aqueous solution was varied from 6.0 to 11.0 within HCl or 1 N NaOH. The fermentation was carried out at 37°C to study the effect on enzyme production. The fermentation was carried out at various temperatures such as 25°C, 30°C, 37°C and 45°C to study their effect on enzyme production keeping all other conditions at the optimum level.

Entrapment of cells in non-toxic alginate is one of the simplest, cheapest and most frequently used of immobilized (Iso *et al.*, 2001). Sodium alginate and Calcium chloride were used to prepare the alginate beads containing the whole cells. Sodium alginate solution (3%wt/vol) was prepared by dissolving sodium alginate in 100 ml hot water. The contents were stirred vigorously for 10 minute to obtain thick uniform slurry without any undissolved lumps and then sterilized by autoclaving. Both alginate slurry cell suspensions (equivalent to 0.03 gm dry cell weight) were mixed and stirred for 10 minutes to obtain to get a uniform mixture. The slurry was taken into a sterile stringe, added drop-wise into 0.2m $CaCl_2$ solutions from 5cm height and kept for curing at 4°C for 1 hour. The cured beads were washed with sterile water 3 to 4 times. When the beads are not use, these are preserved in normal saline solution in a refrigerator (Sexana *et al.*, 1999).

RESULTS AND DISCUSSION

After 24h incubation, plates were observed for lipase producing colonies based on the clear zone formed around the growth. Growing colonies with blue colour zones were isolated and subsequently screened by double layer by tributrin agar method. Lipase productivity of *Pseudomonas aeroginosa* and *Staphylococcus aureus* was analyzed in molasses and soybean substrate and the S_1, S_2 are named as *Candida albicans* and *Saccharomyces cerevisiae*. The lipase activity was estimed (Sakakai *et al.*, 2001) and the results are presented in Table 1. The effect of pH and temperature on lipase production during growth were studied using mineral medium described.

Table 1 : Assay of enzyme activity

Sr. No.	Organisms	Substrate	Enzyme activity unit /ml/min
1.	*Candida albicans*	Molasses	0.30±0.03
		Soybean	0.27±0.07
2.	*Saccharomyces cerevisiae*	Molasses	0.20±0.03

The lipase productivity was optimized by changing initial pH of the medium was adjusting from 5 to 10 after fermentation the lipase activities were estimated and the results are presented in (Table 2). The culture inoculated medium was incubated at different range from 20-45°C and the results are presented in (Table 3). The lipase enzyme productivity was estimated in the immobilized *Candida albicans* and *Saccharomyces cerevisiae*. The immobilized organism was inoculated in the fermented medium. After fermentation of the lipases productivity was studied and the results are presented in (Table 4).

Table 2 : Lipase productivity of *Candida albicans* in various pH range

Sr. No.	Substrate	pH range	Enzyme activity U/ml/min
1.		4	0.17±0.04
2.		5	0.32±0.041
3.	Molasses	6	0.41±0.50
4.		7	0.37±0.09
5.		8	0.21±0.06
6.		9	0.11±0.03
7.		10	0.9±0.02

Table 3 : Lipase productivity of *Candida albicans* in various temperature

Sr. No.	Substrate	Temperature	Enzyme activity U/ml/min
1.		25	0.13±0.09
2.		30	0.18±0.02
3.	Molasses	35	0.23±0.07
4.		40	0.42±0.11
5.		45	0.31±0.12
6.		50	0.22±0.01

Table 4 : Lipase enzyme productivity in Immobilized cell

Sr. No.	Test immunoorganism cells	Enzyme activity Free	Immobilized
1.	*Candida albicans*	0.35±0.01	0.49± 0.11
2.	*Saccharomyces cerevisiae*	0.27± 0.22	0.25±0.1

In the present study lipase production *Candida albicans* and *Saccharomyces cerevisiae* were estimated in various substrates such as molasses and soybean extract high lipase productivity observed in molasses using *Candida albicans*. Physiochemical conditions were analyzed for the productivity of lipase enzyme in the Soya using *Candida albicans*, in this growth and lipase production was found to be optimal between pH ranges of 7-8 (0.41±0.37 µ/ml/min) temperature maximum at 35ºC±1(0.42 µ/ml/min). The optimum pH and temperature determined in this study are in agreement with the finding of lipase by different microbial strain. The optimal production of lipase by *Bacillus* was also reported (Shibatani *et al*., 2000) at pH 8.0. The enzyme productivity was analyzed in the free and immobilized *Candida albicans* and *Saccharomyces cerevisiae*. In this study the maximum lipase, productivity was observed in immobilized *Candida albicans* and *Saccharomyces cerevisiae* (0.51±0.28µ/ml/min). Batch and repeated batch (fed batch type) experimentation had been carried out for lipase production using immobilized *C. rugosa* cell in expensed bed reactors, having with enriched medium (Soumanou and Bornscheuer, 2003). *R. arrhizus* cells have been immobilized on polyurethane foams and effect of parameters like glucose concentration, pH, inoculums size and agitator speed has been studies on lipase production positive effect of corn oil as induces on the process and the storage stability has been investigated (Sexana *et al.*, 1999). Finally it was concluded that the isolated *Candida albicans* could be used for commercial production of lipase in molasses substrate. This lipase provides great opportunity in biotechnology of which has important industrial significance.

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